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Prediction of PIK3CA mutations from cancer gene expression data

--Manuscript Draft--

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Full Title:	Prediction of PIK3CA mutations from cancer gene expression data
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Keywords:	PIK3CA; targeted therapy; biomarker; machine learning; The Cancer Genome Atlas; pan-cancer analysis; gene expression; predict modeling; penalized logistic regression
Abstract:	Breast cancers with PIK3CA mutations can be treated with PIK3CA inhibitors in hormone receptor-positive HER2 negative subtypes. We applied a supervised elastic net penalized logistic regression model to predict mutations from gene expression data. This regression approach was applied to predict modeling using the TCGA pan-cancer dataset. Approximately 10,000 cases were available for PIK3CA mutation and mRNA expression data. In 10-fold cross-validation, the model with $\lambda = 0.01$ and $\alpha = 1.0$ (ridge regression) showed the best performance, in terms of area under the receiver operating characteristic (AUROC). The final model was developed with selected hyper-parameters using the entire training set. The training set AUROC was 0.93, and the test set AUROC was 0.84. The area under the precision-recall (AUPR) of the training set was 0.66, and the test set AUPR was 0.39. Cancer types were the most important predictors. Both insulin like growth factor 1 receptor (IGF1R) and the phosphatase and tensin homolog (PTEN) were the most significant genes in gene expression predictors. Our study suggests that predicting genomic alterations using gene expression data is possible, with good outcomes.
Order of Authors:	Jun Kang
	Ahwon Lee
	Youn Soo Lee
Opposed Reviewers:	
Response to Reviewers:	Reviewer #1: Comment 1 This is a regulated regression analysis to find out the most significant variable(s) and that is kept in the final model. This is a very concise article and lack of detail methodology. Response Thank you for your constructive feedback considering the lack of detailed methodology. TCGA is a widely used public data of cancer genomics. The detail of the TCGA pan-cancer data is described in the reference. For the method of prediction modeling, we tried to follow guidelines for developing and reporting machine learning predictive models in biomedical research. We add a supplementary figure to help understand hyperparameter tuning. Luo, Wei, Dinh Phung, Truyen Tran, Sunil Gupta, Santu Rana, Chandan Karmakar, Alistair Shilton, et al. “Guidelines for Developing and Reporting Machine Learning Predictive Models in Biomedical Research: A Multidisciplinary View.” Journal of Medical Internet Research 18, no. 12 (2016): e323. https://doi.org/10.2196/jmir.5870. Comment 2

It has been known for quite some times that PIK3CA GOF mutation is very common (in fact next to TP53) in solid tumors. It is also known that PIK3CA is very much related to PTEN and IGF1R signaling.
The finding is not new and the rationale for this article is not very clear.

Response

Thank you for your opinion. Our study aims to build the PIK3CA mutation prediction model not to search for important variables. Because the penalized logistic regression model is highly interpretable, we were able to find significant variables like IGF1R and PTEN. But this findings of significant variables is not the primary purpose of this study. The purpose of this study is to investigate the *PIK3CA* mutation prediction performance of machine learning models. The purpose of the study is further described in the manuscript.

Comment 3

More importantly, this type of article is not suitable for PLOS ONE audience. Authors may consider to some bio-informatics or bio-statistics journal.

Response

Thank you for your suggestion. Since machine learning modeling is complex and has begun to be widely used relatively recently, the audience may lack an understanding of detailed methods. However, our study used a widely used data set (TCGA) and modeling framework (R tidymodels package). We believe our research will benefit audiences interested in applying machine learning to patient care. We also believe that publishers targeting a broad audience are publishing predictive model studies using machine learning.

Reviewer #2:

Comment 1

The authors present a succinct study on the prediction of PIK3CA mutations from gene expression data. This study applies an elastic net penalized logistic regression classifier to the cancer genome atlas (TCGA) pan-cancer gene expression dataset, a method that was previously established for detecting RAS pathway activation. The methods used and the results presented in the figures appear to be appropriate for the work performed. Both the AUROC and AUPRC demonstrate predictive performance well above baseline. Limitations of the approach used were also appropriately discussed.

Response

Thank you for your opinion.

Comment 2

It may be questionable why PIK3CA mutation prediction from mRNA expression is useful when targeted sequencing panels can assay these mutations directly, but it has been proposed elsewhere that clinical transcriptomics may add important functional or phenotypic information.

Response

As you pointed out, the clinical utility of PIK3CA mutation prediction from mRNA expression is unclear because most direct genomic tests are more specific and sensitive than predictive models. Our prediction model is not an application that is immediately applicable to a cancer patient for the detection of PIK3CA mutation. It is not known how it will be used, but finding out the mutation prediction performance using gene expression data could play a role in advancing machine learning to be helpful in patient treatment. We discussed further the limitations of this study in the

	manuscript. Comment 3 Overall this work demonstrates that machine learning approaches can predict PIK3CA mutation status from gene expression data with a reasonably good level of performance. Response Thank you for your opinion.
Additional Information:	
Question	**Response**
Financial Disclosure Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the submission guidelines for detailed requirements. View published research articles from *PLOS ONE* for specific examples. This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate. **Unfunded studies** Enter: *The author(s) received no specific funding for this work.* **Funded studies** Enter a statement with the following details: • Initials of the authors who received each award • Grant numbers awarded to each author • The full name of each funder • URL of each funder website • Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript? • **NO** - Include this sentence at the end of your statement: *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.* • **YES** - Specify the role(s) played. * typeset	The author(s) received no specific funding for this work.
Competing Interests	NO authors have competing interests

Use the instructions below to enter a competing interest statement for this submission. On behalf of all authors, disclose any competing interests that could be perceived to bias this work—acknowledging all financial support and any other relevant financial or non-financial competing interests. This statement **will appear in the published article** if the submission is accepted. Please make sure it is accurate. View published research articles from *PLOS ONE* for specific examples. **NO authors have competing interests** Enter: *The authors have declared that no competing interests exist.* **Authors with competing interests** Enter competing interest details beginning with this statement: *I have read the journal's policy and the authors of this manuscript have the following competing interests: [insert competing interests here]* * typeset	
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The data underlying the results presented in the study are available from (include the name of the third party and contact information or URL). • This text is appropriate if the data are owned by a third party and authors do not have permission to share the data. * typeset	
Additional data availability information:	

Dear, Editor-in-Chief

We are submitting a research article of "Prediction of PIK3CA mutations from cancer gene expression data" written by Jun Kang. The article is submitted to be considered for publication as an original article in the PLOS ONE.

In this manuscript, we tried to build a PIK3CA mutation prediction model from gene expression data. PIK3CA inhibitors are used for patients with breast cancer having a PIK3CA mutation. The machine learning approach can help to find breast cancer patients who will benefit from the PIK3CA inhibitors. We adopt a machine learning approach that had been developed by The Cancer Genome Atlas Research Network. Our model shows good prediction performance with 0.84 test set AUROC. We believe our findings are meaningful in terms of building baseline research data for building a machine learning model as a biomarker.

The manuscript has been written according to the guidelines provided by the PLOS ONE. This or similar material has not been previously published and will not be submitted by me or my colleagues to any other publication before its appearance in the PLOS ONE.

Thank you.

With regards,

Youn Soo Lee, M.D., Ph.D.

Professor

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Prediction of *PIK3CA* mutations from cancer gene expression data

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Abstract

Breast cancers with *PIK3CA* mutations can be treated with *PIK3CA* inhibitors in hormone receptor-positive HER2 negative subtypes. We applied a supervised elastic net penalized logistic regression model to predict *PIK3CA* mutations from gene expression data. This regression approach was applied to predict modeling using the TCGA pan-cancer dataset. Approximately 10,000 cases were available for *PIK3CA* mutation and mRNA expression data. In 10-fold cross-validation, the model with $\lambda = 0.01$ and $\alpha = 1.0$ (ridge regression) showed the best performance, in terms of area under the receiver operating characteristic (AUROC). The final model was developed with selected hyper-parameters using the entire training set. The training set AUROC was 0.93, and the test set AUROC was 0.84. The area under the precision-recall (AUPR) of the training set was 0.66, and the test set AUPR was 0.39. Cancer types were the most important predictors. Both *insulin like growth factor 1 receptor* (*IGF1R*) and the *phosphatase and tensin homolog* (*PTEN*) were the most significant genes in gene expression predictors. Our study suggests that predicting genomic alterations using gene expression data is possible, with good outcomes.

Introduction

Targeted therapy has become a standard treatment for many cancer patients, however the approach requires a test for a specific cancer genomic alteration, to treat patients. Several direct genomic alteration tests have been developed and proven for their clinical utility to treat patients [1].

Machine learning approaches can be applied to detect genomic alterations. Machine learning algorithms can build prediction models from a large number of predictors, such as radiomic features [3], pathology image [4] or gene expression data [5]. Because most direct genomic tests are more specific and sensitive than predictive models, machine learning approaches may have limited roles in clinical practice, however, machine learning approaches are ideal when direct tests are unavailable or fail.

RAS pathway activation predictions have been performed using gene expression data [5]. Authors used data from The Cancer Genome Atlas (TCGA), with a supervised elastic net penalized logistic regression classifier, with stochastic gradient descent. Their model performance was 84% with an area under the receiver operating characteristic (AUROC) curve, and 63% with an area under the precision-recall (AUPR) curve. Importantly, these authors suggested their approach could be applied to other genomic alterations.

Breast cancer having *PIK3CA* mutations can be treated using PIK3CA inhibitors, in hormone receptor-positive HER2 negative subtypes [7]. The *PIK3CA* mutation is the second most common driver mutation after *TP53*, and is most frequently detected in endometrial carcinoma (45%), followed by breast invasive carcinoma (24%), cervical squamous cell carcinoma, endo-cervical adenocarcinoma (20%) and colon adenocarcinoma (16%) [8].

PIK3CA encodes the p110α catalytic subunit of phosphatidylinositol 3′-kinase (PI3K). PI3K is a protein kinase that phosphorylates phosphatidylinositol 4,5-biphosphate (PIP_2) to generate phosphatidylinositol 3,4,5-triphosphate (PIP_3). The phosphatase and tensin homolog (*PTEN*) converts PIP_2 to PIP_3 in contrast to PI3K. PIP_3 is a second messenger that activates protein kinase B (AKT), which is a serine/threonine-specific protein kinase. AKT inhibits apoptosis and promotes cell proliferation [9].

We applied a supervised elastic net penalized logistic regression model to predict *PIK3CA* mutations. We wanted to ascertain whether this prediction model approach could be applied not only to RAS pathway activation, but also to *PIK3CA* mutation predictions. The purpose of this study is to investigate the *PIK3CA* mutation prediction performance of machine learning models.

Materials and Methods

Dataset

We used the TCGA pan-cancer dataset. TCGA archives the following; exome sequencing, gene expression, DNA methylation, protein expression, and clinical data from > 10,000 cancer samples across 33 common cancer types. The TCGA dataset is publically available. *PIK3CA* mutation data was extracted using cgdsr rpackage [10]. Gene expression data was downloaded from the National Cancer Institute (NCI)'s Genomic Data Commons (GDC) website. This archives data for TCGA (https://gdc.cancer.gov/about-data/publications/pancanatlas). Gene expression in the TCGA pan-cancer dataset is batch-corrected with normalization.

The target variable was *PIK3CA* mutation status. *PIK3CA* status was considered positive when the case had the following *PIK3CA* variants (C420R, E542K, E545A, E545D, E545G, E545K, Q546E, Q546R, H1047L, H1047R, H1047Y) which were the target variants of the Therascreen *PIK3CA* RGQ PCR Kit, Qiagen, Hilden, Germany. This kit was approved as a companion diagnostics test to treat with PIK3CA inhibitor by the United States Food and Drug Administration.

Modeling process

To narrow down potential predictors, genes with a large median absolute deviation (> third-quartiles) were selected. Thirty three cancer type dummy variables were included in predictor variables. We split three-quarters of the dataset into the training set and one quarter into the test set. Yeo-Johnson transformation was performed to correct skewness. Centering and scaling were also performed. All preprocessing was performed using the recipe r package [11]. Penalized logistic regression was applied to prediction modeling. Ten-fold cross-validation with target variable stratification was performed over the hyper-parameter grid: λ $\{10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 10^{0}\}$, α {0.0, 0.25, 0.5, 0.75, 1.0}. Lambda (λ) is a penalty scaling parameter and alpha (α) is a mixing parameter of penalty function ($(1-\alpha)/2\|\beta\|_2^2 + \alpha\|\beta\|_1$) [12].

Assessing model performance

Model performance was evaluated using AUROC and AUPR curve approaches. The AUPR approach is more informative than AUROC for imbalanced datasets [13]. The modeling process and assessing model performance were performed with the tidymodels rpackage [14].

Results

Dataset summary

10,845 cases were available for both *PIK3CA* mutation and mRNA expression data. 5,128 out of 20,502 genes were included in the modeling process, after filtering for median absolute deviation, as described in the modeling process method. The prevalence rate for *PIK3CA* mutation was 0.11 in all cases. The *PIK3CA* mutation prevalence rate in each cancer type varied. The median prevalence rate of *PIK3CA* mutation for each cancer type was 0.03 (range 0–0.33) (Figure 1).

Selecting model and performance estimation

For 10-fold cross-validation, the model with $\lambda = 0.01$ and $\alpha = 1.0$ (ridge regression) showed the best performance in terms of AUROC (S1 Figure). The final model was trained with the selected hyper-parameters with the entire training set. The training set AUROC was 0.93 and the test set AUROC was 0.84. The AUPR of the training set was 0.66 and the test set AUPR was 0.39 (Figure 2A).

Performance of each cancer type

Because *PIK3CA* mutation prevalence varied across cancer types, the performance of each cancer type was investigated. The AUROC and AUPR were positively correlated between the training sets and test sets in cancer type sub-analysis (Figure 2B). The AUPR was high in cancer types with high *PIK3CA* mutation rates such as colon, breast and uterus cancer types. The AUROC did not correlate with *PIK3CA* mutation rates of each cancer type (Figure 2C).

Important predictors

The top 30 important predictors are shown (Figure 3). The coefficient is the parameter of the predictor which represents the effect of the predictor on prediction. *Insulin like growth factor 1 Receptor* (*IGF1R*) mRNA expression was the strongest negative predictor, and *PTEN* was the strongest positive predictor. Both *IGF1R* and *PTEN* are key players in the tyrosine kinase pathway [9,15]. The cancer types were important predictors. Some cancer types including uterine carcinosarcoma (UCS), bladder urothelial carcinoma (BLCA), pancreatic adenocarcinoma (PAAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC) were the strongest predictors.

Discussion

Our model showed good performance in predicting *PIK3CA* mutations in various cancer types. Our data suggested that the supervised elastic net penalized logistic regression model could be applied not only to the RAS activation pathway, but also to other genomic alterations. Both the RAS activation pathway and *PIK3CA* mutations are key,

common cancer genomic alterations. Because they exert significant effect on gene expression in cancer cells, prediction from gene expression data can be good. However, the supervised elastic net penalized logistic regression model cannot be generalized or applied to other genomic alterations which have have a weak effect on gene expression.

Prediction modeling from the TCGA pan-cancer dataset can be limiting in terms of data preprocessing. The gene expression data is processed by between-sample normalization to remove batch effects. If the model has been trained from between-sample normalization, a new sample cannot be exactly processed with normalization which was done on trainset. A model based on gene expression from the TCGA pan-cancer dataset has limitation in terms of data preprocessing. It is necessary to develop a processing method that is independent of a dataset, to apply gene expression data to the prediction model.

Our *PIK3CA* prediction model was similar to the RAS activation prediction model in terms of AUROC (0.84). However the AUPR of our model was lower than the RAS activation model (0.39 versus 0.63). The reason for our lower AUPR may be explained by an imbalanced dataset that has the low prevalence rate of *PIK3CA* mutations [5]. The model for RAS activation trained with cancer types with more than 0.05 prevalence of RAS activation to avoid imbalance classification problem. We included all cancer types in our modeling process. The lower prevalence rate of target variables meant our dataset had a lower AUPR baseline. In the sub-analysis performance of each cancer type, the cancer types with higher *PIK3CA* mutation rates showed better AUPRs.

Our model included cancer types as predictors, and they were stronger predictors than gene expression. The varying prevalence of PIC3CA mutations across cancer types may be a reason for the strong predictive power of cancer types.

Some significant gene expression predictors were closely related to the PTEN-PI3K pathway. *PTEN* and *IGFR1R* were the strongest gene expression predictors, which has negative and positive predictive powers. *IGF1R* is a tyrosine kinase receptor that activates PI3K [15], and *PTEN* is an important regulator of PIP_3 by dephosphorylating PIP_3 [9].

Several studies have attempted to predict genomic alterations from gene expression data [6,16]. A study investigated *PIK3CA* mutation predictions using gene-expression signatures which is a sum of the average of the logarithmic gene expression. The model showed good performance AUROC 0.71 in an independent test set [6,16]. Another study predicted copy number alterations with gene expression, using a multinomial logistic regression model with least absolute shrinkage and selection operator (LASSO) parameters [17]. The prediction of the 1p/19q codeletion was very good, with an AUROC of 0.997, and gene-level predictions were good, with an AUROC of 0.75 [17]. A logistic regression model was used for *MYCN Proto-Oncogene, BHLH Transcription Factor* (*MYCN*) gene amplification in neuroblastoma [18].

The clinical utility of PIK3CA mutation prediction from mRNA expression is unclear because most direct genomic tests are more specific and sensitive than predictive models. Our prediction model is not an application that is immediately applicable to a cancer patient for detection of PIK3CA mutation. It is not known how it will be used, but finding out the mutation prediction performance using gene expression data could play a role in advancing machine learning to be helpful in patient treatment.

Our study suggested that the prediction of genomic alterations using gene expression data was possible, with good performance. However, improved performances are required for clinical tests, and the standardization of generation processing of gene expression data is also needed.

Figure legends

- Figure 1. Prevalence rate of *PIK3CA* mutations across cancer types. Cancer type abbreviations are explained in the S1 Appendix.
- Figure 2. Summary of modeling results. (A) Left: receiver operating characteristic (ROC) curve. Right: precision-recall (PR) curve of training set and test set. The horizontal green line is the *PIK3CA* mutation rate (0.11) (B) Correlation between training set and test set of the area under the receiver operating characteristic curve (AUROC), and the area under the precision-recall curve (AUPR) among cancer types. The gray band is the 95% confidence interval. Abbreviations are explained in the S1 Appendix. (C) Correlations between the *PIK3CA* mutation rate of the AUROC, and the AUPR.
- Figure 3. Coefficient model. (A) Top 30 high mRNA coefficients. (B) Cancer type coefficients. Cancer types abbreviations are explained in the S1 Appendix.

Supporting information

- S1 Appendix. Abbreviations of cancer type.
- S1 Figure. Hyperparameter tuning and performance assessment in 10-fold cross-validation resampling. The x-axis is a penalty scaling parameter: λ $\{10^{-5}$, 10^{-4},10^{-3},10^{-2},10^{-1}, $10^{0}\}$, color is mixture hyperparameter of penalty function: α $\{0.0, 0.25, 0.5, 0.75, 1.0\}$. y-axis is estimates of area under the receiver operating characteristic (AUROC) using 10-fold cross-validation resampling.

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Figure1

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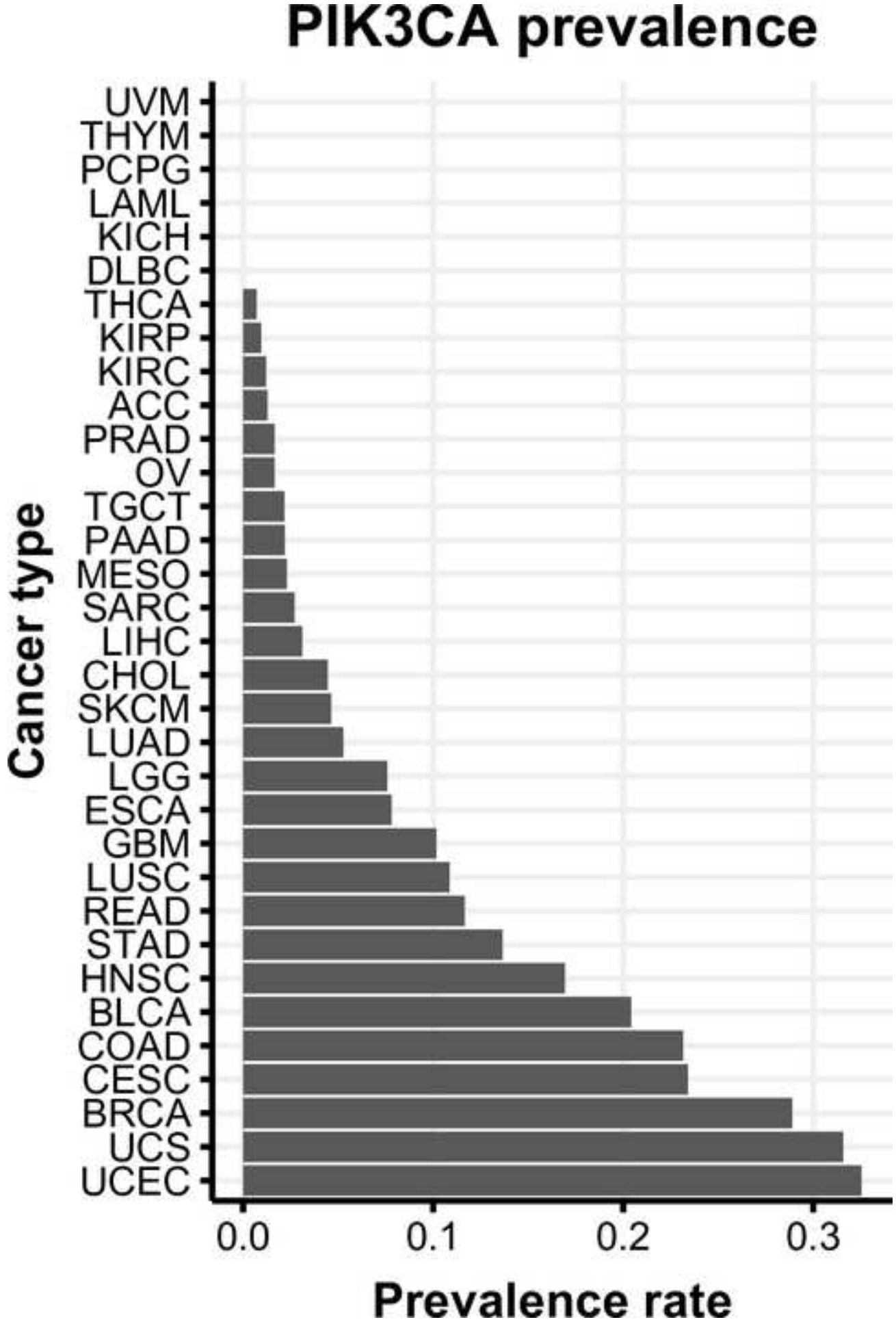

Figure2

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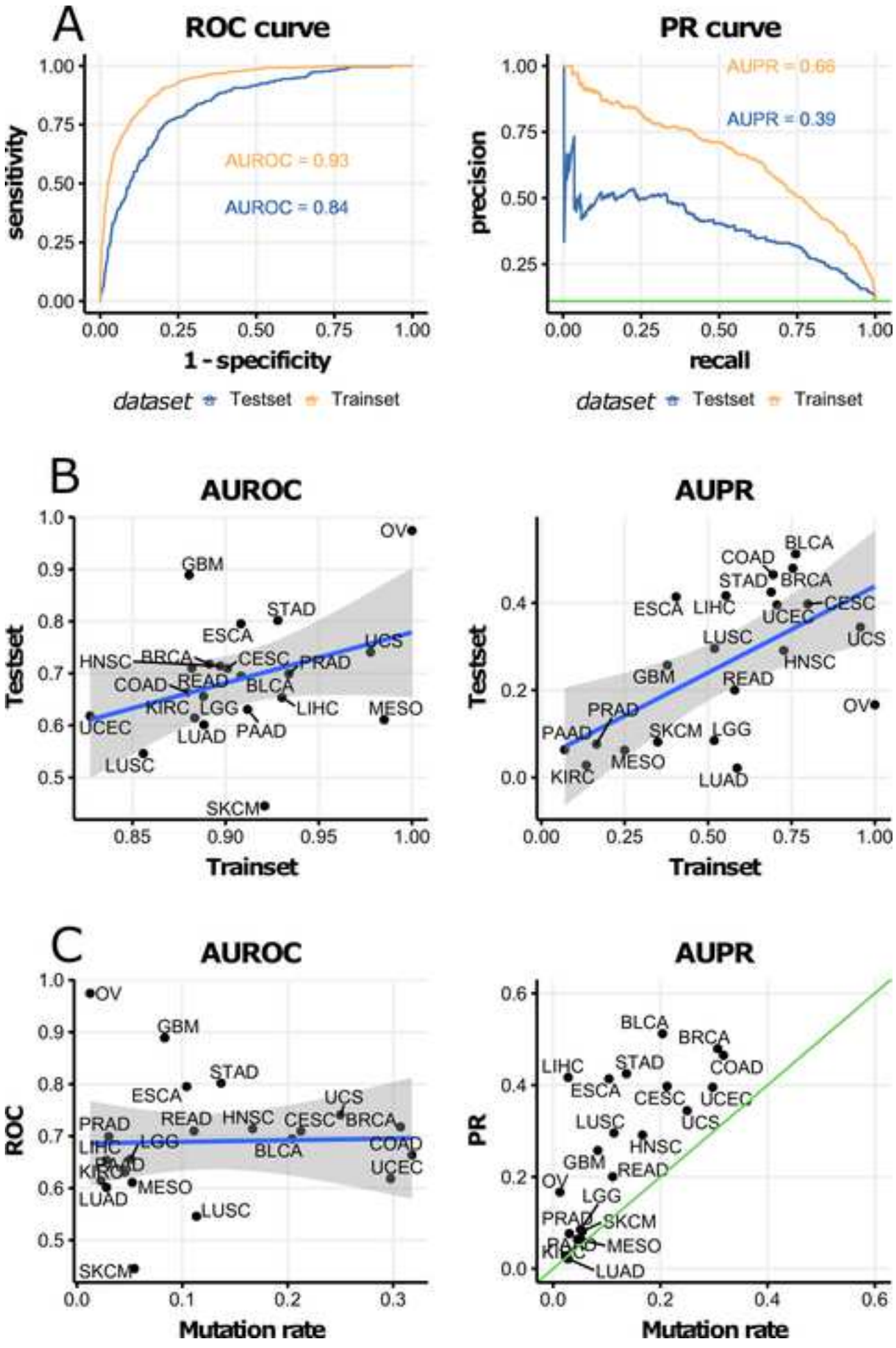

Figure3

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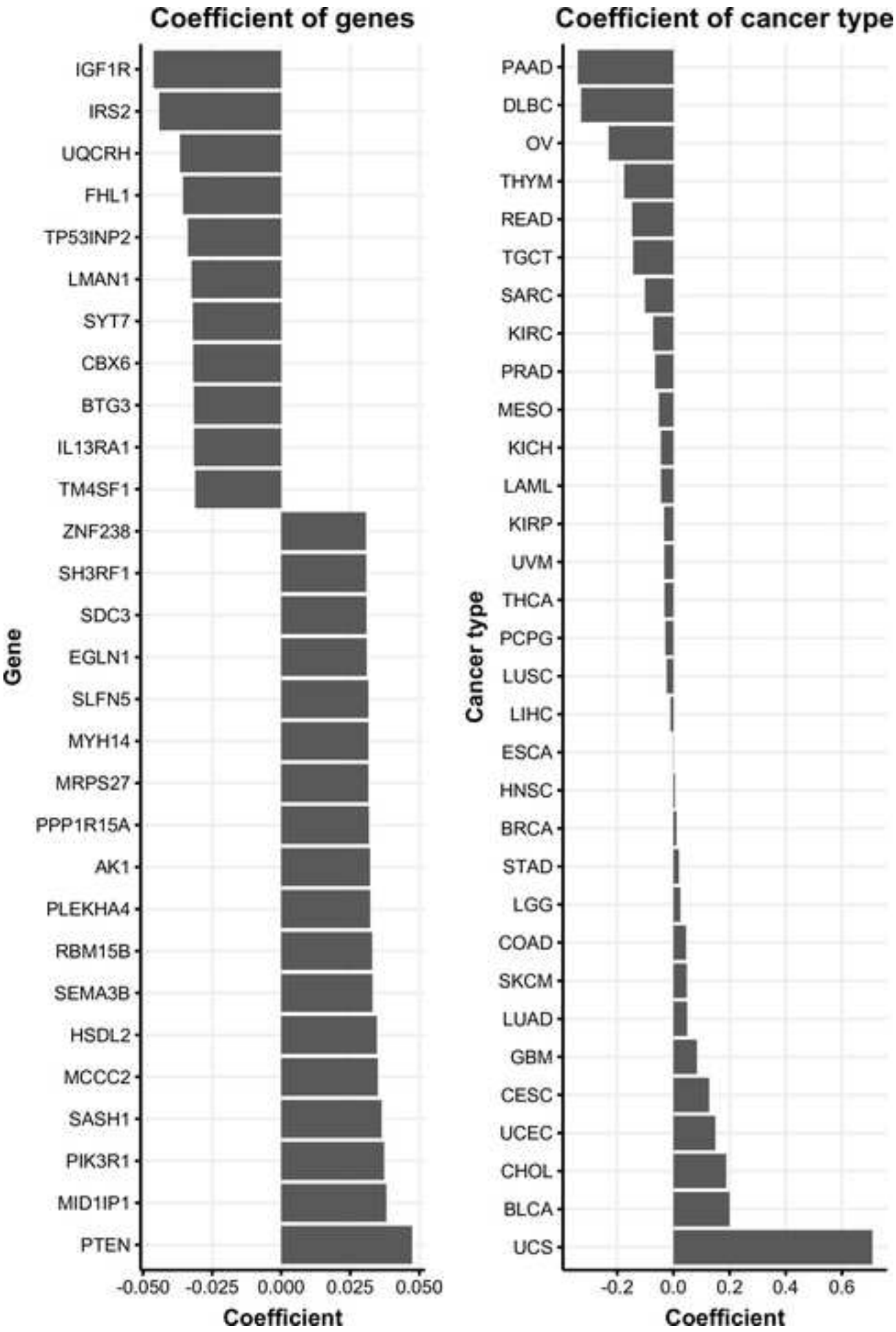

Prediction of *PIK3CA* mutations from cancer gene expression data

Breast cancers with *PIK3CA* mutations can be treated with *PIK3CA* inhibitors in hormone receptor-positive HER2 negative subtypes. We applied a supervised elastic net penalized logistic regression model to predict *PIK3CA* mutations from gene expression data. This regression approach was applied to predict modeling using the TCGA pan-cancer dataset. Approximately 10,000 cases were available for *PIK3CA* mutation and mRNA expression data. In 10-fold cross-validation, the model with λ = 0.01 and α = 1.0 (ridge regression) showed the best performance, in terms of area under the receiver operating characteristic (AUROC). The final model was developed with selected hyper-parameters using the entire training set. The training set AUROC was 0.93, and the test set AUROC was 0.84. The area under the precision-recall (AUPR) of the training set was 0.66, and the test set AUPR was 0.39. Cancer types were the most important predictors. Both *insulin like growth factor 1 receptor* (*IGF1R*) and the *phosphatase and tensin homolog* (*PTEN*) were the most significant genes in gene expression predictors. Our study suggests that predicting genomic alterations using gene expression data is possible, with good outcomes.

Introduction

Targeted therapy has become a standard treatment for many cancer patients, however the approach requires a test for a specific cancer genomic alteration, to treat patients. Several direct genomic alteration tests have been developed and proven for their clinical utility to treat patients [1].

Machine learning approaches can be applied to detect genomic alterations. Machine learning algorithms can build prediction models from a large number of predictors, such as radiomic features [3], pathology image [4] or gene expression data [5]. Because most direct genomic tests are more specific and sensitive than predictive models, machine learning approaches may have limited roles in clinical practice, however, machine learning approaches are ideal when direct tests are unavailable or fail.

RAS pathway activation predictions have been performed using gene expression data [5]. Authors used data from The Cancer Genome Atlas (TCGA), with a supervised elastic net penalized logistic regression classifier, with stochastic gradient descent. Their model performance was 84% with an area under the receiver operating characteristic (AUROC) curve, and 63% with an area under the precision-recall (AUPR) curve. Importantly, these authors suggested their approach could be applied to other genomic alterations.

Breast cancer having *PIK3CA* mutations can be treated using PIK3CA inhibitors, in hormone receptor-positive HER2 negative subtypes [7]. The *PIK3CA* mutation is the second most common driver mutation after *TP53*, and is most frequently detected in endometrial carcinoma (45%), followed by breast invasive carcinoma (24%), cervical squamous cell carcinoma, endo-cervical adenocarcinoma (20%) and colon adenocarcinoma (16%) [8].

PIK3CA encodes the p110α catalytic subunit of phosphatidylinositol 3′-kinase (PI3K). PI3K is a protein kinase that phosphorylates phosphatidylinositol 4,5-biphosphate (PIP_2) to generate phosphatidylinositol 3,4,5-triphosphate (PIP_3). The phosphatase and tensin homolog (*PTEN*) converts PIP_2 to PIP_3 in contrast to PI3K. PIP_3 is a second messenger that activates protein kinase B (AKT), which is a serine/threonine-specific protein kinase. AKT inhibits apoptosis and promotes cell proliferation [9].

We applied a supervised elastic net penalized logistic regression model to predict *PIK3CA* mutations. We wanted to ascertain whether this prediction model approach could be applied not only to RAS pathway activation, but also to *PIK3CA* mutation predictions. The purpose of this study is to investigate the *PIK3CA* mutation prediction performance of a supervised elastic net penalized logistic regression model.

Commented [c1]: R1-2

Materials and Methods

Dataset

We used the TCGA pan-cancer dataset. TCGA archives the following; exome sequencing, gene expression, DNA methylation, protein expression, and clinical data from > 10,000 cancer samples across 33 common cancer types. The TCGA dataset is publically available. *PIK3CA* mutation data was extracted using cgdsr rpackage [10]. Gene expression data was downloaded from the National Cancer Institute (NCI)'s Genomic Data Commons (GDC) website. This archives data for TCGA (https://gdc.cancer.gov/about-data/publications/pancanatlas). Gene expression in the TCGA pan-cancer dataset is batch-corrected with normalization.

The target variable was *PIK3CA* mutation status. *PIK3CA* status was considered positive when the case had the following *PIK3CA* variants (C420R, E542K, E545A, E545D, E545G, E545K, Q546E, Q546R, H1047L, H1047R, H1047Y) which were the target variants of the Therascreen *PIK3CA* RGQ PCR Kit, Qiagen, Hilden, Germany. This kit was approved as a companion diagnostics test to treat with PIK3CA inhibitor by the United States Food and Drug Administration.

Modeling process

To narrow down potential predictors, genes with a large median absolute deviation (> third-quartiles) were selected. Thirty three cancer type dummy variables were included in predictor variables. We split three-quarters of the dataset into the training set and one quarter into the test set. Yeo-Johnson transformation was performed to correct skewness. Centering and scaling were also performed. All preprocessing was performed using the recipe r package [11]. Penalized logistic regression was applied to prediction modeling. Ten-fold cross-validation with target variable stratification was performed over the hyper-parameter grid: λ $\{10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 10^{0}\}$, α {0.0, 0.25, 0.5, 0.75, 1.0}. Lambda (λ) is a penalty scaling parameter and alpha (α) is a mixing parameter of penalty function $((1-\alpha)/2 \parallel \beta \parallel_2^2 + \alpha \parallel \beta \parallel_1)$ [12].

Assessing model performance

Model performance was evaluated using AUROC and AUPR curve approaches. The AUPR approach is more informative than AUROC for imbalanced datasets [13]. The modeling process and assessing model performance were performed with the tidymodels rpackage [14].

Results

Dataset summary

10,845 cases were available for both *PIK3CA* mutation and mRNA expression data. 5,128 out of 20,502 genes were included in the modeling process, after filtering for median absolute deviation, as described in the modeling process method. The prevalence rate for *PIK3CA* mutation was 0.11 in all cases. The *PIK3CA* mutation prevalence rate in each cancer type varied. The median prevalence rate of *PIK3CA* mutation for each cancer type was 0.03 (range 0–0.33) (Figure 1).

Selecting model and performance estimation

For 10-fold cross-validation, the model with $\lambda = 0.01$ and $\alpha = 1.0$ (ridge regression) showed the best performance in terms of AUROC. (S1 Figure). The final model was trained with the selected hyper-parameters with the entire training set. The training set AUROC was 0.93 and the test set AUROC was 0.84. The AUPR of the training set was 0.66 and the test set AUPR was 0.39 (Figure 2A).

Commented [c2]: R1-1

Performance of each cancer type

Because *PIK3CA* mutation prevalence varied across cancer types, the performance of each cancer type was investigated. The AUROC and AUPR were positively correlated between the training sets and test sets in cancer type sub-analysis (Figure 2B). The AUPR was high in cancer types with high *PIK3CA* mutation rates such as colon, breast and uterus cancer types. The AUROC did not correlate with *PIK3CA* mutation rates of each cancer type (Figure 2C).

Important predictors

The top 30 important predictors are shown (Figure 3). The coefficient is the parameter of the predictor which represents the effect of the predictor on prediction. *Insulin like growth factor 1 Receptor* (*IGF1R*) mRNA expression was the strongest negative predictor, and *PTEN* was the strongest positive predictor. Both *IGF1R* and *PTEN* are key players in the tyrosine kinase pathway [9,15]. The cancer types were important predictors. Some cancer types including uterine carcinosarcoma (UCS), bladder urothelial carcinoma (BLCA), pancreatic adenocarcinoma (PAAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC) were the strongest predictors.

Discussion

Our model showed good performance in predicting *PIK3CA* mutations in various cancer types. Our data suggested that the supervised elastic net penalized logistic regression model could be applied not only to the RAS activation pathway, but also to other genomic alterations. Both the RAS activation pathway and *PIK3CA* mutations are key, common cancer genomic alterations. Because they exert significant effect on gene expression in cancer cells, prediction from gene expression data can be good. However, the supervised elastic net penalized logistic regression model cannot be generalized or applied to other genomic alterations which have have a weak effect on gene expression.

Prediction modeling from the TCGA pan-cancer dataset can be limiting in terms of data preprocessing. The gene expression data is processed by between-sample normalization to remove batch effects. If the model has been trained from between-sample normalization, a new sample cannot be exactly processed with normalization which was done on trainset. A model based on gene expression from the TCGA pan-cancer dataset has limitation in terms of data preprocessing. It is necessary to develop a processing method that is independent of a dataset, to apply gene expression data to the prediction model.

Our *PIK3CA* prediction model was similar to the RAS activation prediction model in terms of AUROC (0.84). However the AUPR of our model was lower than the RAS activation model (0.39 versus 0.63). The reason for our lower AUPR may be explained by an imbalanced dataset that has the low prevalence rate of *PIK3CA* mutations [5]. The model for RAS activation trained with cancer types with more than 0.05 prevalence of RAS activation to avoid imbalance classification problem. We included all cancer types in our modeling process. The lower prevalence rate of target variables meant our dataset had a lower AUPR baseline. In the sub-analysis performance of each cancer type, the cancer types with higher *PIK3CA* mutation rates showed better AUPRs.

Our model included cancer types as predictors, and they were stronger predictors than gene expression. The varying prevalence of PIC3CA mutations across cancer types may be a reason for the strong predictive power of cancer types.

Some significant gene expression predictors were closely related to the PTEN-PI3K pathway. *PTEN* and *IGFR1R* were the strongest gene expression predictors, which has negative and positive predictive powers. *IGF1R* is a tyrosine kinase receptor that activates PI3K [15], and *PTEN* is an important regulator of PIP_3 by dephosphorylating PIP_3 [9].

Several studies have attempted to predict genomic alterations from gene expression data [6,16]. A study investigated *PIK3CA* mutation predictions using gene-expression signatures which is a sum of the average of the logarithmic gene expression. The model showed good performance AUROC 0.71 in an independent test set [6,16]. Another study predicted copy number alterations with gene expression, using a multinomial logistic regression model with least absolute shrinkage and selection operator (LASSO) parameters [17]. The prediction of the 1p/19q codeletion was very good, with an AUROC of 0.997, and gene-level predictions were good, with an AUROC of 0.75 [17]. A logistic regression model was used

for *MYCN Proto-Oncogene, BHLH Transcription Factor* (*MYCN*) gene amplification in neuroblastoma [18].

The clinical utility of PIK3CA mutation prediction from mRNA expression is unclear because most direct genomic tests are more specific and sensitive than predictive models. Our prediction model is not an application that is immediately applicable to a cancer patient for the detection of PIK3CA mutation. It is not known how it will be used, but finding out the mutation prediction performance using gene expression data could play a role in advancing machine learning to be helpful in patient treatment.

Commented [c3]: R2-2

Our study suggested that the prediction of genomic alterations using gene expression data was possible, with good performance. However, improved performances are required for clinical tests, and the standardization of generation processing of gene expression data is also needed.

Figure legends

- Figure 1. Prevalence rate of *PIK3CA* mutations across cancer types. Cancer type abbreviations are explained in the S1 Appendix.
- Figure 2. Summary of modeling results. (A) Left: receiver operating characteristic (ROC) curve. Right: precision-recall (PR) curve of training set and test set. The horizontal green line is the *PIK3CA* mutation rate (0.11) (B) Correlation between training set and test set of the area under the receiver operating characteristic curve (AUROC), and the area under the precision-recall curve (AUPR) among cancer types. The gray band is the 95% confidence interval. Abbreviations are explained in the S1 Appendix. (C) Correlations between the *PIK3CA* mutation rate of the AUROC, and the AUPR.
- Figure 3. Coefficient model. (A) Top 30 high mRNA coefficients. (B) Cancer type coefficients. Cancer types abbreviations are explained in the S1 Appendix.

Supporting information

- S1 Appendix. Abbreviations of cancer type.
- S1 Figure. Hyperparameter tuning and performance assessment in 10-fold cross-validation resampling. The x-axis is a penalty scaling parameter: λ {10^{-5}, 10^{-4},10^{-3},10^{-2},10^{-1}, 10^{0}}, color is mixture hyperparameter of penalty function: α {0.0, 0.25, 0.5, 0.75, 1.0}. y-axis is estimates of area under the receiver operating characteristic (AUROC) using 10-fold cross-validation resampling.

Commented [c4]: R1-1

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Response to Reviewers

Dear, Editor-in-Chief, PLOS ONE

Thank you for considering our manuscript entitled "Prediction of PIK3CA mutations from cancer gene expression data (PONE-D-20-22669)." We are really happy to hear that the manuscript is considering for publication with a revision. We considered all suggestions that were raised by the reviewers and tried to answer to all queries to the best of our capability. In our annotated version, we indicated what we responded to reviewers' suggestions by using Track Change function with marginal notes and symbols (e.g., R1-1 indicates response to comment #1 of Reviewer #1). Our specific responses are as follows:

Comments to the Author

1. Is the manuscript technically sound, and do the data support the conclusions?

The manuscript must describe a technically sound piece of scientific research with data that supports the conclusions. Experiments must have been conducted rigorously, with appropriate controls, replication, and sample sizes. The conclusions must be drawn appropriately based on the data presented.

Reviewer #1: Partly

Reviewer #2: Yes

2. Has the statistical analysis been performed appropriately and rigorously?

Reviewer #1: I Don't Know

Reviewer #2: Yes

3. Have the authors made all data underlying the findings in their manuscript fully available?

The PLOS Data policy requires authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception (please refer to the Data Availability Statement in the manuscript PDF file). The data should be provided as part of the manuscript or its supporting information, or deposited to a public repository. For example, in addition to summary statistics, the data points behind means, medians and variance measures should be available. If there are restrictions on publicly sharing data—e.g. participant privacy or use of data from a third party—those must be specified.

Reviewer #1: Yes

Reviewer #2: Yes

4. Is the manuscript presented in an intelligible fashion and written in standard English?

PLOS ONE does not copyedit accepted manuscripts, so the language in submitted articles must be clear, correct, and unambiguous. Any typographical or grammatical errors should be corrected at revision, so please note any specific errors here.

Reviewer #1: Yes

Reviewer #2: Yes

5. Review Comments to the Author

Please use the space provided to explain your answers to the questions above. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. (Please upload your review as an attachment if it exceeds 20,000 characters)

Reviewer #1:

Comment 1

This is a regulated regression analysis to find out the most significant variable(s) and that is kept in the final model. This is a very concise article and lack of detail methodology.

Response

Thank you for your constructive feedback considering the lack of detailed methodology. TCGA is a widely used public data of cancer genomics. The detail of the TCGA pan-cancer data is described in the reference. For the method of prediction modeling, we tried to follow guidelines for developing and reporting machine learning predictive models in biomedical research. We add a supplementary figure to help understand hyperparameter tuning.

Luo, Wei, Dinh Phung, Truyen Tran, Sunil Gupta, Santu Rana, Chandan Karmakar, Alistair Shilton, et al. "Guidelines for Developing and Reporting Machine Learning Predictive Models in Biomedical Research: A Multidisciplinary View." Journal of Medical Internet Research 18, no. 12 (2016): e323. https://doi.org/10.2196/jmir.5870.

Comment 2

It has been known for quite some times that PIK3CA GOF mutation is very common (in fact next to TP53) in solid tumors. It is also known that PIK3CA is very much related to PTEN and IGF1R signaling. The finding is not new and the rationale for this article is not very clear.

Response

Thank you for your opinion. Our study aims to build the PIK3CA mutation prediction model not to search for important variables. Because the penalized logistic regression model is highly interpretable, we were able to find significant variables like IGF1R and PTEN. But this findings of significant variables is not the primary purpose of this study. The purpose of this study is to investigate the *PIK3CA* mutation prediction performance of machine learning models. The purpose of the study is further described in the manuscript.

Comment 3

More importantly, this type of article is not suitable for PLOS ONE audience. Authors may consider to some bio-informatics or bio-statistics journal.

Response

Thank you for your suggestion. Since machine learning modeling is complex and has begun to be widely used relatively recently, the audience may lack an understanding of detailed methods. However, our study used a widely used data set (TCGA) and modeling framework (R tidymodels package). We believe our research will benefit audiences interested in applying machine learning to patient care. We also believe that publishers targeting a broad audience are publishing predictive model studies using machine learning.

Reviewer #2:

Comment 1

The authors present a succinct study on the prediction of PIK3CA mutations from gene expression data. This study applies an elastic net penalized logistic regression classifier to the cancer genome atlas (TCGA) pan-cancer gene expression dataset, a method that was previously established for detecting RAS pathway activation. The methods used and the results presented in the figures appear to be appropriate for the work performed. Both the AUROC and AUPRC demonstrate predictive performance well above baseline. Limitations of the approach used were also appropriately discussed.

Response

Thank you for your opinion.

Comment 2

It may be questionable why PIK3CA mutation prediction from mRNA expression is useful when targeted sequencing panels can assay these mutations directly, but it has been proposed elsewhere that clinical transcriptomics may add important functional or phenotypic information.

Response

As you pointed out, the clinical utility of PIK3CA mutation prediction from mRNA expression is unclear because most direct genomic tests are more specific and sensitive than predictive models. Our prediction model is not an application that is immediately applicable to a cancer patient for detection of PIK3CA mutation. It is not known how it will be used, but finding out the mutation prediction performance using gene expression data could play a role in advancing machine learning to be helpful in patient treatment. We discussed further the limitations of this study in the manuscript.

Comment 3

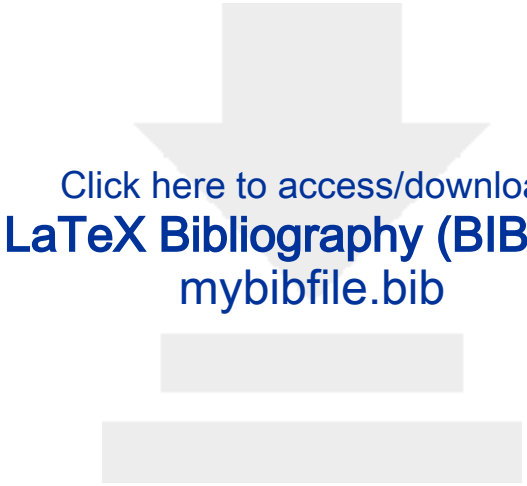
Overall this work demonstrates that machine learning approaches can predict PIK3CA mutation status from gene expression data with a reasonably good level of performance.

Response

Thank you for your opinion.

Thank you again for reconsidering our manuscript. We think we did our best to revise faithfully our previous manuscript in line with the indications that were raised by the reviewer. We hope the revised version would meet with your, and the reviewer's approval and be finally accepted by the PLOS ONE.

With best wishes and respectfulness, Youn Soo Lee, MD



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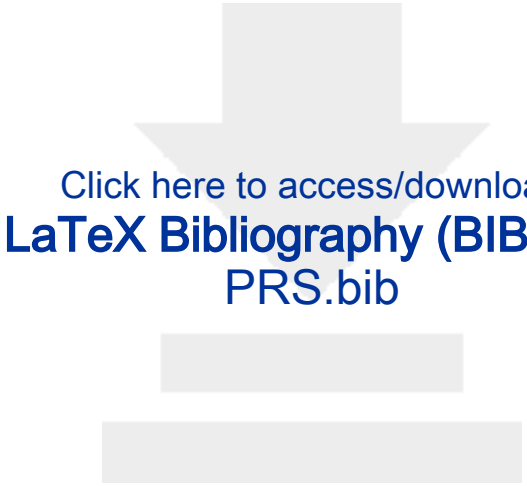

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